ENVIRONMENTAL PROTECTION AGENCY
Publications Distribution Section
Route 8, Box 116, Hwy. 70, West
Raleigh, North Carolina 27607

Official Business

POSTAGE AND FEES PAID
ENVIRONMENTAL PROTECTION AGENCY

If your address is incorrect, please change on the above label; tear off; and return to the above address.
If you do not desire to continue receiving this technical report series, CHECK HERE ☐ ; tear off label, and return it to the above address.

WATER POLLUTION CONTROL RESEARCH SERIES • 13030 ELY 08/71-9
REC-R2-71-9
DWR NO.174-12

BIO-ENGINEERING ASPECTS OF AGRICULTURAL DRAINAGE

SAN JOAQUIN VALLEY, CALIFORNIA

THE EFFECTS OF AGRICULTURAL WASTE WATER TREATMENT ON ALGAL BIOASSAY RESPONSE

AUGUST 1971

VIRONMENTAL PROTECTION AGENCY • RESEARCH AND MONITORING

BIO-ENGINEERING ASPECTS OF AGRICULTURAL DRAINAGE
SAN JOAQUIN VALLEY, CALIFORNIA

The Bio-Engineering Aspects of Agricultural Drainage reports describe the results of a unique interagency study of the occurrence of nitrogen and nitrogen removal treatment of subsurface agricultural wastewaters of the San Joaquin Valley, California.

The three principal agencies involved in the study are the Water Quality Office of the Environmental Protection Agency, the United States Bureau of Reclamation, and the California Department of Water Resources.

Inquiries pertaining to the Bio-Engineering Aspects of Agricultural Drainage reports should be directed to the author agency, but may be directed to any one of the three principal agencies.

THE REPORTS

It is planned that a series of twelve reports will be issued describing the results of the interagency study.

There will be a summary report covering all phases of the study.

A group of four reports will be prepared on the phase of the study related to predictions of subsurface agricultural wastewater quality -- one report by each of the three agencies, and a summary of the three reports.

A group of three reports will be prepared to include: (1) the techniques to remove nitrogen in drainage effluent during transport, (2) the possibilities of reducing nitrogen in drainage water by on-farm practices, and (3) desalination of subsurface agricultural waste waters.

This report, "THE EFFECTS OF AGRICULTURAL WASTE WATER TREATMENT ON ALGAL BIOASSAY RESPONSE," has been prepared with three other reports on the treatment methods studied and on the biostimulatory testing of the treatment plant effluent. A summary of the three basic reports will also be prepared.

BIO-ENGINEERING ASPECTS OF AGRICULTURAL DRAINAGE
SAN JOAQUIN VALLEY, CALIFORNIA

THE EFFECTS OF AGRICULTURAL WASTE WATER TREATMENT
ON ALGAL BIOASSAY RESPONSE

Prepared by the

Environmental Protection Agency
William D. Ruckelshaus, Administrator

The agricultural drainage study was conducted
under the direction of:

Paul De Falco, Jr., Regional Administrator, Region 9
ENVIRONMENTAL PROTECTION AGENCY
100 California Street, San Francisco, California 94111

Robert J. Pafford, Jr., Regional Director, Region 2
UNITED STATES BUREAU OF RECLAMATION
2800 Cottage Way, Sacramento, California 95825

John R. Teerink, Deputy Director
CALIFORNIA DEPARTMENT OF WATER RESOURCES
1416 Ninth Street, Sacramento, California 95814

REC-R2-71-9
DWR No. 174-12
13030 ELY 08/71-9
August 1971

For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402 - Price $1.00

REVIEW NOTICE

This report has been reviewed by the U.S. Bureau of Reclamation and the California Department of Water Resources, and has been approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Bureau of Reclamation, or the California Department of Water Resources.

The mention of trade names or commercial products does not constitute endorsement or recommendation for use by either of the two federal agencies or the California Department of Water Resources.

ABSTRACT

Laboratory bioassay experiments were performed to test the effect on algal growth of agricultural waste water before and after the waste water had been subjected to two different nitrogen removal processes. The waste waters were added in various percentages to San Joaquin River Delta water for bioassay. The algal growth throughout time was monitored by chlorophyll fluorescence techniques. The fluorescence measurements showed logarithmic growth similar to the type usually observed in the Delta water over the vernal growth period.

The laboratory experiments gave positive statistical evidence that the untreated agricultural waste water would promote substantial algal growth above that of the San Joaquin River controls. Both nitrogen removal processes were equally effective in lowering the algal growth to that of the Delta water controls as long as the nitrate-nitrogen level in each removal system had been lowered to approximately 2 mg N/l or less.

Key words: Algal blooms - control, bioassay - algal, chlorophyll, denitrification, fluorometry, tile drainage.

BACKGROUND

This report is one of a series which presents the findings of intensive interagency investigations of practical means to control the nitrate concentration in subsurface agricultural waste water prior to its discharge into other water. The primary participants in the program are the Water Quality Office of the Environmental Protection Agency, the United States Bureau of Reclamation, and the California Department of Water Resources, but several other agencies also are cooperating in the program. These three agencies initiated the program because they are responsible for providing a system for disposing of subsurface agricultural waste water from the San Joaquin Valley of California and protecting water quality in California's water bodies. Other agencies cooperated in the program by providing particular knowledge pertaining to specific parts of the overall task.

The need to ultimately provide subsurface drainage for large areas of agricultural land in the western and southern San Joaquin Valley has been recognized for some time. In 1954, the Bureau of Reclamation included a drain in its feasibility report of the San Luis Unit. In 1957, the California Department of Water Resources initiated an investigation to assess the extent of salinity and high ground water problems and to develop plans for drainage and export facilities. The Burns-Porter Act, in 1960, authorized San Joaquin Valley drainage facilities as part of the State Water Facilities.

The authorizing legislation for the San Luis Unit of the Bureau of Reclamation's Central Valley Project, Public Law 86-488, passed in June 1960, included drainage facilities to serve project lands. This Act required that the Secretary of Interior either provide for constructing the San Luis Drain to the Delta or receive satisfactory assurance that the State of California would provide a master drain for the San Joaquin Valley that would adequately serve the San Luis Unit.

Investigations by the Bureau of Reclamation and the Department of Water Resources revealed that serious drainage problems already exist and that areas requiring subsurface drainage would probably exceed 1,000,000 acres by the year 2020. Disposal of the drainage into the Sacramento-San Joaquin Delta near Antioch, California, was found to be the least costly alternative plan.

Preliminary data indicated the drainage water would be relatively high in nitrogen. The then Federal Water Quality

Administration conducted a study to determine the effect of discharging such drainage water on the quality of water in the San Francisco Bay and Delta. Upon completion of this study in 1967, the Administration's report concluded that the nitrogen content of untreated drainage waters could have significant adverse effects upon the fish and recreation values of the receiving waters. The report recommended a three-year research program to establish the economic feasibility of nitrate-nitrogen removal.

As a consequence, the three agencies formed the Interagency Agricultural Waste Water Study Group and developed a three-year cooperative research program which assigned specific areas of responsibility to each of the agencies. The scope of the investigation included an inventory of nitrogen conditions in the potential drainage areas, possible control of nitrates at the source, prediction of drainage quality, changes in nitrogen in transit, and methods of nitrogen removal from drain waters including biological-chemical processes and desalination.

TABLE OF CONTENTS

FIGURES

TABLES

CHAPTER I - CONCLUSIONS

1. San Joaquin River algal bioassay responses were related to the nitrate and algal content. Summer and early fall bioassay samples had a high initial algal growth and a low nitrate concentration; little growth occurred in the laboratory.

   Winter and spring samples had a low initial algal population and a high nitrate concentration; large algal growth occurred during the course of the bioassays.

2. Mixtures of San Joaquin River water and untreated agricultural drainage consistently stimulated algal growth, regardless of the season.

3. The bioassay responses of mixtures of treated agricultural drainage with San Joaquin River water showed that nitrogen removal from agricultural drainage is definitely effective in reducing biostimulation.

4. Effluents from algal pond and anaerobic denitrification treatment systems produced statistically equal bioassay responses when compared on the basis of the nitrate and nitrite remaining in the effluent. In general, algal bioassay responses varied inversely with the efficiency of nitrogen removal. High terminal chlorophyll bioassay concentrations were associated with inefficient removal of nitrogen from the waste water.

5. When inorganic nitrogen was below 2 mg/l in treated drainage, algal growth responses of the mixture of treated drainage and San Joaquin River water produced responses similar to those of San Joaquin River controls.

6. Algal growth responses observed in untreated agricultural waste water were reproduced when nitrate-nitrogen was re-added to the treated waste waters bringing the levels back to those found in the original waste water.

CHAPTER II - INTRODUCTION

Algal bioassay methods were used to evaluate nutrient removal processes under study at the Interagency Agricultural Waste Water Treatment Center (IAWWTC), Firebaugh, California. This study was conducted as part of a project to reduce algal growth when agricultural waste water is mixed with San Joaquin Delta water. Upon adding waste water or nitrogen and phosphorus to San Joaquin River water, analyses were made comparing bioassay algal growth responses in treated and untreated agricultural waste water. The experimental growth responses were similar to algal growth observed in the San Joaquin River (Figure 1). The proposed agricultural tile drain will empty into the San Joaquin River near Antioch. The first part of this report is devoted to the chemical analyses of both the San Joaquin River water collected near Antioch and the treated and untreated agricultural waste water. This section is followed by the algal bioassay responses to nutrient chemical addition and to agricultural waste water.

Much of the data has been normalized to overcome the variable growth response of the control medium, the San Joaquin River water. Normalization consisted of dividing all of the data from each bioassay by the bioassay value for the San Joaquin River water for that date. In this way, results can be logically compared or summarized when the responses for the basic medium are equalized for all bioassays. This normalization technique was applied to the waste water treated chlorophyll growth responses and growth rates (u_b, day^{-1}) and to the algal pond and bacterial filter bioassay efficiency comparisons. The final part of the main report relates seasonal variations in chlorophyll and nitrate concentrations in the San Joaquin River water.

The detailed analyses of all the experiments utilizing bacterial filter, algal pond, and untreated agricultural waste water are given in the appendices along with sections reporting bioassay algal species changes, and bioassay correlations between cell count, chlorophyll, and nitrate concentrations.

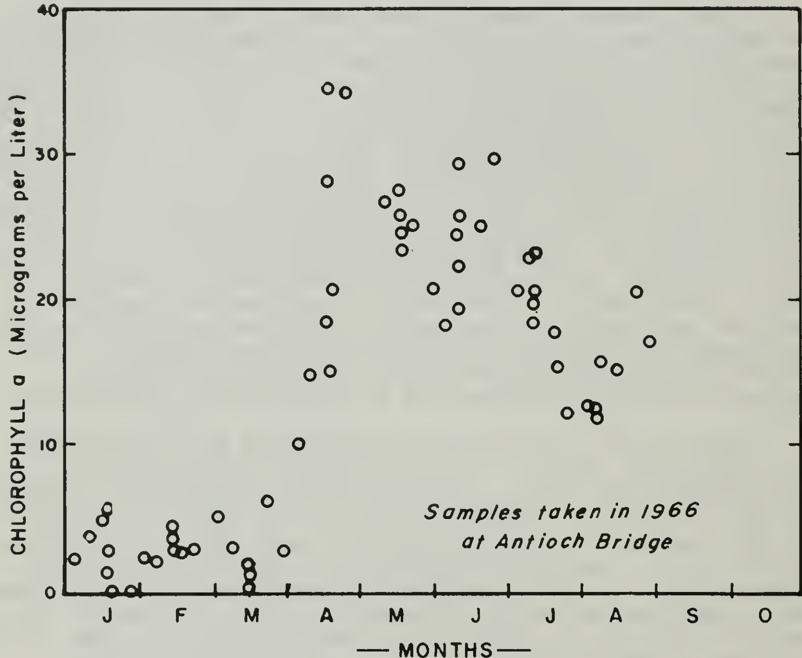

Figure 1. Seasonal Chlorophyll Variations in San Joaquin River Water

AGRICULTURAL WASTE WATER STUDIES
SAN JOAQUIN VALLEY, CALIFORNIA

ENVIRONMENTAL PROTECTION AGENCY
REGION IX
SAN FRANCISCO, CALIFORNIA

CHAPTER III - METHODS AND PROCEDURES

WATER COLLECTION

The agricultural waste water was collected in polyethylene containers on the afternoon prior to the beginning of each bioassay, while the San Joaquin River water was collected early the next day. Personnel from the Interagency Agricultural Waste Water Treatment Center stopped at Antioch, California to collect San Joaquin River water on their way to the EPA laboratory at Alameda, California. Usually the Antioch samples were collected approximately four hours before the bioassay was started. The algal pond waste water was prefiltered through GFA glass filter pads at Firebaugh immediately after collection, reducing algal concentration to that found in the bacterial filter and anaerobic pond waters. This filtration also corresponded to the algal harvesting from the pond waters which is an integral part of the algal stripping process.

EXPERIMENTAL PROCEDURE

Untreated agricultural drainage, algal pond, anaerobic pond, or bacterial filter waters were added separately to San Joaquin River water in dilutions ranging from 1 percent to 50 percent of the total sample volume. These percentage additions were chosen because they approximated the probable future concentrations of waste water in the San Joaquin River. Undiluted waste water was used in some initial experiments to test for specific nutrient deficiencies in these waters. Each sample for bioassay was prepared in triplicate. A sample of 300 ml was placed in a 500 ml Erlenmeyer flask which was then plugged with a foam rubber stopper. An aliquot was saved for nitrate analysis.

In another series of bioassays, inorganic nutrients (KNO_3 or KH_2PO_4) were added to the San Joaquin River water to determine the results of nitrate or phosphate, alone or combined, on the algal growth. Before any assay was started, chlorophyll *a* concentrations were measured.

Incubation of the cultures was in a 20° C $\pm$ 1° C constant temperature walk-in refrigerator. The flasks were put on plate glass shelves with cool-white fluorescent lights mounted one inch below each shelf (Figure 2). Approximately 600 ft-candles of light reached the bottom of the flasks. Flasks were incubated from 5 to 15 days until algae reached maximum growth. A blower and a system or ducts forced

Figure 2. Flasks in Culture Box

air constantly through the space between the fluorescent tubes and the plate glass and through the area above the glass shelves. (Without this forced-air system, the fluorescent tubes cause a slight warming of the plate glass shelves.) Since the blower was running continuously, the air temperature was kept nearly constant.

In the first series of bioassays (12/13/68-4/4/69), algal growth was monitored by extracted chlorophyll fluorescence. The cultures were subsampled on the day they were set up and on alternate days thereafter. Fifty-milliliter subsamples were taken from each culture. Following filtration through GFC glass fiber pads, they were macerated in a cell homogenizer, and chlorophyll was extracted by 90 percent acetone. The method is described in detail by Bain (1969).

Algal growth in the second series of bioassays (6/18/69 - 11/17/69) was measured daily, except for weekends, by direct fluorescence of a small subsample (10 ml) taken from each flask being incubated. The foam rubber stoppers were replaced by ones of hard rubber and the flasks were shaken. Readings were taken on a Turner Fluorometer equipped with a Corning CS 5-60 primary filter and a Corning CS 2-60 secondary filter. Normally the 30x scale was used. The subsamples were discarded after fluorescence readings since the flasks contained enough volume for many readings without appreciable change in the surface-to-volume ratio. The readings were taken until algal fluorescence had reached a plateau or had started to decline, which usually occurred in 5 to 7 days. At the termination of the experiment, the water from the replicate flasks was combined and used for NO_3 determinations.

Direct fluorescence readings were converted to chlorophyll concentrations for each sample. These readings were followed by filtration and 90 percent-acetone extraction of larger volumes of water from 1-liter flasks containing the same media as the smaller flasks. Extracted samples were measured for optical densities at specific wavelengths following chlorophyll determination procedures recommended by Strickland and Parsons (1965). Analyses for nitrate - nitrogen, nitrite-nitrogen, organic nitrogen, ammonia, inorganic phosphorus, and total phosphorus followed the procedures listed in the FWPCA Methods for Chemical Analyses of Water and Wastes, (1969).

EXPERIMENTAL DESIGN

The experimental design was factorial, permitting an analysis of variance of the data followed by a multiple range test, if the variance was significant. The multiple range test used was that of Student-Neuman-Keuls (Steel and Torrie, 1960). Three growth parameters were derived from the flask culture growth curves: (1) increase in chlorophyll concentration, (2) maximum chlorophyll concentration, and (3) maximum observed growth rate (μ_b, day^{-1}).

CHAPTER IV - RESULTS

CHEMICAL ANALYSES

San Joaquin River Water

Chemical analyses for the San Joaquin River water used as the basic medium in the algal bioassays are given in Table 1. The collection dates correspond to bioassay experiment dates and cover a period of one year, from December 1968 through November 1969.

Changes in most of the measured parameters appear moderate. Total P has a value of 0.65 mg/l for June, but all other samples have concentrations between 0.07-0.16 mg P/l. Changes in PO_4-P and organic N concentrations are small. The highest total inorganic N values (NH_3-N plus NO_3-N) occur in the January and February samples, 1.10 and 0.95 mg N/l respectively, with low values of slightly more than 0.10 mg N/l present during the summer months. The highest total inorganic N to PO_4-P ratio was approximately 11:1 in the January 4 sample; the lowest was 2:1 in July and August.

Agricultural Drain Waste Water

The chemical analyses of agricultural waste water samples added to San Joaquin River water are listed in Tables 2 and 3. The determinations include NO_3-N, NO_2-N, PO_4-P, and soluble organic nitrogen. Any NH_3-N in the samples was reported as part of the soluble organic nitrogen. On the basis of many determinations, the NH_3-N level is 0.10 mg N/l or less in the algal pond and untreated agricultural tile drain waste water and less than 1.5 mg N/l in the bacterial filter water (Sword, 1970).

Untreated agricultural tile drainage is high in NO_3-N concentrations, 9.2 mg N/l to 22.5 mg N/l. Nitrate-nitrogen concentrations also vary in the treated agricultural waste water because samples showed different levels of nitrogen removal. The highest effluent nitrate value was 5.1 mg N/l produced by bacterial filter No. 19 on August 18, 1969. The measurement 9.2 mg NO_3-N in the original untreated waste water indicated that less than half of the nitrate was removed. Some of the treated samples had trace concentrations of nitrate ($<$0.10 mg N/l) showing a high efficiency of NO_3-N removal.

TABLE 1. - CHEMICAL ANALYSIS OF SAN JOAQUIN RIVER WATER COLLECTED IN THE VICINITY OF ANTIOCH

Chemical	1968	1969											
	12/13	1/4	1/28	2/14	3/10	4/4	6/18	7/24	8/18	9/7	9/29	10/20	11/17
Alkalinity $CaCO_3$,mg/1	68	-	-	59	-	-	46	54	68	118	68	69	78
PO_4-P,mgP/1	0.07	0.07	.02	0.08	0.07	0.07	0.07λ	0.07	0.07	0.06	0.4	0.03	0.06
Total P,mgP/1	0.07	-	0.09	0.11	0.08	-	0.65	0.12	0.13	0.10	0.12	0.14	0.16
Org N,mg N/1	0.54	0.46	0.47	-	-	-	0.37	0.31	0.42	0.26	0.35	0.41	0.32
NH_3,mg N/1	0.15	0.17	0.28	0.08	0.15	0.12	0.09	0.10	<0.08	0.09	<0.08	0.08	0.09
NO_3,mg N/1	0.43	0.61	0.82	0.95	0.53	0.22	0.18	0.04	0.05	0.08	-	0.09	0.22
Total inorganic nitrogen, mg N/1	0.58	0.78	1.10	1.13	0.68	0.34	0.27	0.14	0.13	0.17	(0.08)	0.17	0.31

TABLE 2. - CHEMICAL ANALYSES OF AGRICULTURAL TILE DRAIN WASTE WATER UTILIZED IN THE NUTRIENT RE-ADDITION EXPERIMENTS

Date	Chemical Analyses mg/l	Untreated Agricultural Waste Water	Treated Agricultural Waste Water[a] Bacterial Filter		
			No. 14		No. 19
12/13/69	NO_3-N	20.3	1.3		3.0
	NO_2-N	<0.001	1.0		3.7
	Sol.Org-N	0.40	1.94		0.48
			No. 11	No. 13	No. 14
1/4/69	NO_3-N	17.9	0.2	0.6	0.4
	NO_2-N	<0.001	<0.001	0.01	0.04
	Sol.Org-N	0.27	0.39	0.70	0.89
	PO_4-P	0.05	<0.02	<0.02	<0.02
	Total-P	<0.02	<0.02	<0.02	<0.02
			Algal Pond	Bacterial Filter No. 14	Bacterial Filter No. 19
1/28/69	NO_3-N	17.0	1.2	1.1	1.2
	NO_2-N	0.004	5.4	2.22	3.7
	Sol.Org-N	0.39	1.25	0.57	0.59
	PO_4-P	<0.02	0.05	<0.02	<0.02
			No. 19	No. 20	
2/14/69	NO_3-N	19.6	3.2	0.36	
	NO_2-N	0.005	2.80	1.44	
	Sol.Org-N	0.36	1.60	1.48	
	PO_4-P	0.10	0.03	0.12	
				No. 19	No. 20
3/10/69	NO_3-N	18.6	8.0	2.7	<0.10
	NO_2-N	0.01	0.04	2.26	0.12
	Sol.Org-N	0.25	1.17	0.60	0.66
	PO_4-P	0.04	0.02	<0.02	<0.02

a. Identification numbers refer to pilot test unit effluent not to bioassay samples. Some of these units were not identified by numbers.

TABLE 3. - CHEMICAL ANALYSIS OF AGRICULTURAL TILE DRAIN WASTE WATER UTILIZED IN THE COMPARATIVE NITROGEN REMOVAL PROCESS EXPERIMENTS

Date	Chemical Analyses (mg/l)	Untreated Agricultural Waste Water	Treated Agricultural Waste Water: Algal Pond		Treated Agricultural Waste Water: Bacterial Filter	
6/18/69	NO3-N	11.0	<0.10		0.46	
	NO2-N	0.002	<0.001		<0.001	
	Sol.Org-N	0.30	1.06		0.83	
	PO4-P	0.16	0.06		0.06	
7/24/69			No.4			
	NO3-N	10.0	0.15		<0.10	
	NO2-N	0.006	0.019		<0.001	
	Sol.Org-N	0.25	1.15		1.1	
	PO4-P	0.19	<0.02		0.10	
8/18/69			No. 15	No. 14	No. 11	No. 19
	NO3-N	9.2	1.4	3.1	0.5	5.1
	NO2-N	0.02	0.38	0.51	0.001	2.34
	Sol.Org-N	0.04	1.45	1.99	0.90	1.76
	PO4-P	0.12	<0.02	<0.02	<0.02	0.02
9/8/69			No. 6	No. 19	Covered Pond[b]	
	NO3-N	11.5	<0.10	2.2	<0.10	0.5
	NO2-N	0.01	0.02	0.75	<0.001	0.5
	Sol.Org-N	0.04	1.0	1.6	1.13	1.5
	PO4-N	0.07	0.02	0.04	0.03	0.04
9/29/69			No. 5		No. 15	No. 18
	NO3-N	16.3	3.4		0.3	0.6
	NO2-N	0.01	0.24		0.36	0.92
	Sol.Org-N	0.4	1.32		1.0	1.04
	PO4-P	0.08	<0.02		0.03	0.07
10/20/69			No. 6		No. 10	No. 15
	NO3-N	16.5	2.70		<0.10	1.1
	NO2-N	0.01	0.24		0.05	0.35
	Sol.Org-N	0.4	0.95		1.0	0.85
	PO4-P	0.2	1.39[c]		0.15	0.06
11/17/69			No. 14		No. 6	
	NO3-N	22.5	1.80		0.10	
	NO2-N	0.004	0.06		0.06	
	Sol.Org-N	0.4	0.62		1.35	
	PO4-P	0.15	0.07		0.05	

b. An anaerobic - covered pond carrying out the same processes as the bacterial filters.

c. Probably due to experimental phosphorus additions to the pilot algal ponds.

Nitrite concentrations were unusually high in some bacterial filter and algal pond samples taken before June 18, 1969. For example, all of the bacterial filter and algal pond samples collected on January 28, 1968, contained more than 2 mg NO_2-N/l and little more than 1 mg NO_3-N/l. The agricultural tile drain waste water from which the algal pond and bacterial filter samples were derived contained 17.0 mg NO_3-N/l but only 0.004 mg NO_2-N/l indicating that some of the effluent nitrite came from the biological reduction of nitrate.

Inorganic PO_4-P concentrations were usually less than 0.2 mg P/l in the untreated agricultural waste waters and occasionally below the sensitivity of the analytical method used.

ALGAL BIOASSAY RESPONSES

Extracted Fluorescence Experiments

a) Inorganic Phosphorus Additions:

The bioassay response of raw and treated agricultural tile drain waste water, with and without phosphorus additions, is shown in Table 4. These extracted fluorescence values are each the mean of two replicate samples. Since these data are reported in extracted fluorescence units it should be noted that algal cellular chlorophyll concentration and extracted fluorescence are highly correlated (correlation coefficient = 0.99). The fluorescence value in units is approximately 0.5 times the concentration of chlorophyll a in micrograms per liter.

The two samples of raw agricultural waste water (12/13/68 and 1/4/69) had a marked response to inorganic phosphorus additions. Extracted fluorescence of the 12/13/68 sample increased from 220 to 1660 fluorescence units and from 330 to 750 fluorescence units in the 1/4/69 sample.

The bacterial filter effluent exhibited a lower response than the raw waste water. The 1/4/69 samples for bacterial filter Nos. 11, 13, and 14 showed almost no growth relative to that of the original waste water (1, 2, and 1 extracted fluorescence units, respectively).

TABLE 4. - MAXIMUM EXTRACTED FLOURESCENCE OF AGRICULTURAL TILE DRAIN WASTE WATER BIOASSAYS BEFORE AND AFTER INORGANIC PHOSPHORUS ADDITIONS

Sample 100% Concentration	Date	Total Soluble mg N/l	Before P Addition		After P Addition	
			mg PO_4-P/l	Extracted Flourescence	mg PO_4-P/l	Extracted Flourescence
Untreated Agricultural Waste Water	12-13-68	20.70	0.2	220	1.2	1660
	1-4-69	18.17	<0.05	330	<1.05*	750
Bacterial Filter Effluent						
No. 11	1-4-69	0.59	<0.02	1	<0.22+	1
No. 13	1-4-69	1.31	<0.02	2	<0.22	10
No. 14	12-13-68	4.24	<0.02	200	<0.22	232
No. 14	1-4-69	1.33	<0.02	1	<0.22	33
No. 19	12-13-68	7.18	<0.02	56	<0.22	600

* At least 1.00 mg PO_4-P/l
\+ At least 0.20 mg PO_4-P/l

Additions of phosphorus to the bacterial filter effluent brought dramatic algal response only in the No. 19 sample of 12/13/68 (Table 4), where fluorescence increased from 56 to 600 units. This filter had the highest remaining concentration of total soluble nitrogen (7.18 mg N/l) of any listed for 12/13/68 or 1/4/69. Phosphorous limited samples (N:P $>$300) gave the highest growth responses upon the additions of phosphorus.

b) <u>Nitrate-Nitrogen Additions</u>:

Nitrate was added to effluent from algal ponds and bacterial filters to determine whether nitrate re-addition would increase algal growth to values comparable to those of untreated waste water (Table 5).

For all of the responses listed, algal growth in the treated controls was less than that in raw waste water. The addition of nitrate-nitrogen to the bacterial filter and algal pond samples usually increased algal chlorophyll fluorescence above the level found in the original bacterial filter and algal pond effluents. For example, the 10 percent algal pond sample of 1/28/69 had a fluorescent bioassay response of 262 units. Nitrogen additions increased the fluorescence to 370 and 344 units but did not increase fluorescent values to those of the original untreated waste water. The sample containing 10 percent untreated agricultural waste water had a direct fluorescence of 392 units.

Based on the ratios of nitrogen-to-phosphorus, it was noted that nitrogen did not appear to be lacking in the cultures. Phosphorous values include only inorganic phosphorous values, and not organic phosphorus which could have been used by the algae. Increases in fluorescence did not occur after each nitrogen re-addition to the treated waste waters. In the 100 percent bacterial filter samples, as shown by the bacterial filter No. 19 samples of 3/10/69, algal bioassay responses, both with and without nitrogen additions, were very low and almost equal to each other. One probable reason for the lack of response was the few algae contained in the 100 percent bacterial filter effluent.

TABLE 5. - MAXIMUM EXTRACTED FLOURESCENCE OF AGRICULTURAL TILE DRAIN WASTE WATER BIOASSAYS WITH NITROGEN ADDITIONS

SAMPLE AND DATE		% of (x) DRAIN WATER	PO_4-P/1	Before NO_3-N Addition		After 1/2 NO_3-N Addition (d)		After 1 NO_3-N Addition	
				Total Solids mg N/1	Extracted Flourescence	Total Solids mg N/1	Extracted Flourescence	Total Solids mg N/1	Extracted Flourescence
1-28-69									
Agricultural Waste Water		10%	<0.02	2.73	392	---	---	---	---
Treated	AP(e)	10%	0.02	1.78	262	10.28	370	18.78	344
Agricultural	BF(f)-14	10%	<0.02	1.38	341	9.88	396	18.38	380
Waste Water	BF-19	10%	0.02	1.54	346	10.04	356	18.54	392
2-14-69									
Agricultural Waste Water		10%	0.08	3.01	225	---	---	---	---
Treated	AP-19	10%	0.08	1.78	162	11.58	200	21.38	180
Agricultural	AP-19	30%	0.06	3.07	115	12.87	115	22.67	115
Waste Water	AP-20	10%	0.08	1.34	154	11.14	199	20.94	215
	AP-20	30%	0.09	1.78	95	11.58	185	21.38	245
3-10-69		10%	0.07	2.50	400	---	---	---	---
Agricultural Waste Water		100%	0.04	18.86	114	---	---	---	---
	AP	10%	0.06	1.54	300	10.84	355	20.14	440
Treated	BF-19	30%	<0.06	2.14	260	11.44	390	20.74	380
Agricultural	BF-19	100%	0.02	5.56	8	14.86	4	24.16	7
Waste Water	BF-20	30%	<0.06	0.74	200	10.06	230	19.34	205
	BF-20	100%	<0.02	<0.88	2	10.18	30	19.48	1

(x) Percentage of drain waste water of total sample equals drain water plus San Joaquin River water.
(d) The nitrogen was re-added to the treated waste water at concentrations equal to or half of that found in the tile drainage.
(e) AP = Algal Pond
(f) BF = Bacterial Filter

Direct Fluorescence Experiments

a) Cell Mass Changes:

The measured experimental cell mass changes were the increases in chlorophyll and maximum chlorophyll concentrations. These were derived by conversion of direct fluorescence to chlorophyll *a* concentration for the three replicates per sample type. Both variables were used because the samples for comparison did not initially contain equal concentrations of algae; the amount depended mainly on the dilution of the San Joaquin River water. The test samples contained some algae, but most of the algae were present in the San Joaquin River water.

The chemical analyses of the waste water used in the direct fluorescence experiments are given in Table 3. The waste water was added to San Joaquin River water on each experiment date. The analyses for the river water are shown in Table 1.

The complete experimental results from the algal growth comparisons in Appendix A show increases in chlorophyll and maximum chlorophyll concentrations.

b) Summary of Cell Mass Changes:

In five out of seven experiments, the untreated agricultural tile drain waste water consistently gave growth responses above those obtained for the San Joaquin River control (0 percent concentration). Usually the 10 percent and 20 percent concentrations gave equal responses. However, the 1 percent addition of treated agricultural waste water rarely gave growth responses above those of the San Joaquin River control.

The results of the 10 percent and 20 percent bacterial filter and algal pond additions can be understood by relating the growth response to effluent nitrogen. For example, in samples taken on August 18, 1969, (Table 3), inorganic nitrogen in algal ponds Nos. 14 and 15 was 3.61 and 1.78 mg N/l, respectively, and 7.44 mg N/l in bacterial filter No. 19. In contrast, bacterial filter No. 11 had only 0.50 mg N/l of inorganic nitrogen. None of the samples containing filter No. 11 effluent exceeded the control in algal response, whereas all of the other

samples gave growth responses above the controls at one or both of the 10 percent or 20 percent additions (Table A1).

A few of the responses could not be explained on the basis of the nitrogen content of the treated effluents. Both of the treated agricultural waste water samples of July 24, 1969 had a low total inorganic nitrogen content (Table 3). The algal pond effluent contained 0.17 mg N/l while the bacterial filter contained 0.10 mg N/l. Yet algal growth responses of the 20 percent bacterial filter samples exceeded responses from the algal pond (Appendix A). Another example of responses not clarified by the inorganic nitrogen concentrations is shown by effluent samples from filters Nos. 10 and 15 of October 20, 1969. Sample No. 15 had a total inorganic nitrogen content of 1.45 mg N/l, while sample No. 10 totaled $<$ 0.15 mg N/l (Table 3). Bioassay chlorophyll increases for the two bacterial filters effluents, however, were equal in the 10 percent additions. Sample No. 10 exceeded No. 15 in the 20 percent additions by 20.4 to 12.6 mg chlorophyll a per liter (chl a/l).

Bacterial filter effluent contained up to 1.5 mg NH_3-N/l during the summer months but normally contained less than 1.0 mg NH_3-N during other periods (Sword, 1970). Since ammonia was analyzed as part of the soluble organic nitrogen, it was not included in the total inorganic nitrogen values.

In Figure 3, contrary to expectation, the bacterial filter growth response drops below that of the algal pond effluent response. For the same concentrations, the algal pond and agricultural tile drain waste water flasks show growth increasing monatonically with waste water concentration.

c) Normalized Bioassay Chlorophyll Cell Mass Responses:

The variable algal growth in San Joaquin River water (0%, without addition of waste water) indicated that in order to summarize the results, normalization of the data was more logical than utilization of the absolute response values. Normalization was accomplished by dividing the results for each experiment by maximum bioassay growth response of the control water used. Furthermore, bacterial filter and algal pond values were not separated but placed into four categories based on their original NO_3-N plus NO_2-N concentrations:

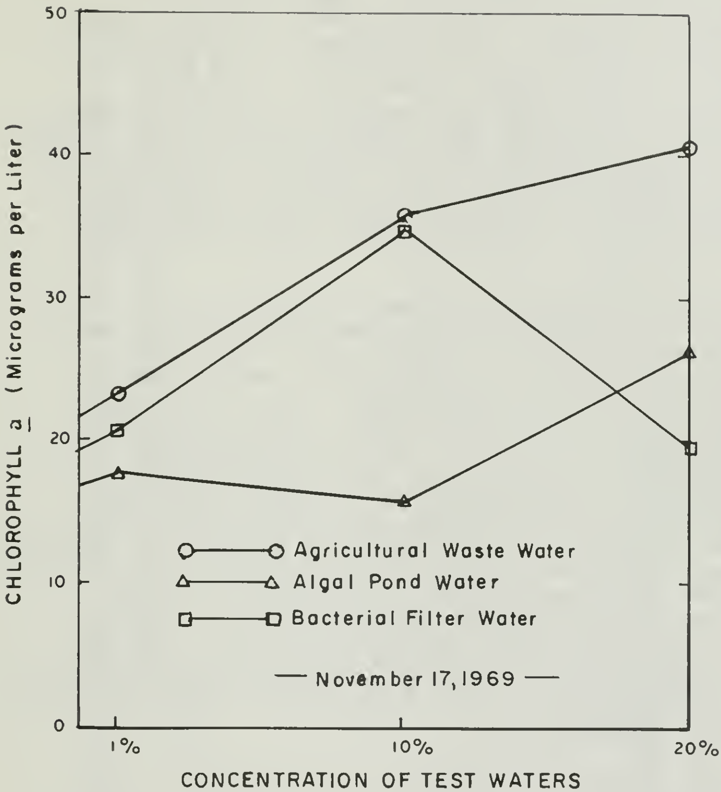

Figure 3. Growth Response Measured by Culture Chlorophyll a

Note: An example of statistical interaction is the decline of algal growth in the bacterial filter 20% concentration compared to increases in the other two 20% dilution samples.

AGRICULTURAL WASTE WATER STUDIES
SAN JOAQUIN VALLEY, CALIFORNIA

ENVIRONMENTAL PROTECTION AGENCY
REGION IX
SAN FRANCISCO, CALIFORNIA

0.13-0.17, 0.46-1.00, 1.45-1.86, and 2.94-7.44 mg N/l. These categories were chosen arbitrarily for statistical evaluation. The untreated agricultural tile drain waste water responses were reported together. Total inorganic nitrogen in the tile drain ranged from 9.22 to 22.50 mg N/l (Table 3). San Joaquin River water contained from 0.13 to 0.27 mg N/l (Table 1). For comparison purposes, the normalized responses for the San Joaquin River water are 1.00. Agricultural tile drain waste water comparisons are included in Table 6 but not in Table 7. The ranges and means of inorganic nitrogen in the original samples are listed under each category.

It is best to utilize the mean value for the inorganic nitrogen in the river water and the waste water to calculate the mixed sample inorganic nitrogen. Using this procedure, a sample made by adding 10 percent treated waste water containing 2.94-7.44 mg N/l to San Joaquin River water would have a final inorganic nitrogen concentration of approximately 0.59 mg N/l. Utilizing the mean values of the San Joaquin River water and of the waste waters, the mean inorganic organic nitrogen value of treated waste water is 4.12 mg N/l. Therefore, 100 ml of this water would contain 0.412 mg N, and 900 ml of San Joaquin River water (0.20 mg N/l, mean value) would contain 0.18 mg N. Adding 0.412 to 0.18 gives a concentration of 0.59 mg N/l for the mixture. Other samples can be calculated in the same manner, i.e. a 20 percent addition of the same waste water to San Joaquin River water would contain 0.98 mg N/l.

Another feature of Table 6 is the bracketing and underlining of values which do not differ significantly from each other at the 95 percent confidence level. For the 1 percent additions, therefore, the values 1.00 through 3.20 are connected by underlining. This means that these responses are statistically equal, regardless of their absolute value. The number 6.63 is significantly higher in value than the others.

Table 6 shows that the 10% and 20% additions of the treated agricultural waste water containing 2.94 - 7.44 mg N/l were the only samples exceeding San Joaquin River water in algal growth.

TABLE 6. - NORMALIZED ALGAL BIOASSAY RESPONSE(g) OF TREATED AND UNTREATED AGRICULTURAL WASTE WATER(h)

	mg N/1 of NO_3-N and NO_2-N in Sample: Range and Mean					
Normalized Response (g)	San Joaquin River	Treated Agricultural Waste Water			Agricultural Waste Water	
Percent Addition	0.13-0.27 0.20	0.10-0.17 0.13	0.46-1.00 0.66	1.45-1.86 1.65	2.94-7.44 4.12	9.22-22.50 13.86
1%	1.00	1.81	1.04	1.29	3.20	6.63
10%	1.00	2.70	1.88	3.91	13.31	27.89
20%	1.00	5.94	3.94	9.36	20.98	29.55
Combined 1%, 10%, and 20% Additions	1.00	3.34	2.29	4.85	12.50	20.95

(g) Normalized Response = $\left\{\frac{\text{Maximum chlorophyll increases in treated water}}{\text{Maximum chlorophyll increases in San Joaquin River water}}\right.$

(h) Samples connected by underlining do not differ in the 95% confidence level

Untreated tile drain waste water gave growth responses above the undiluted San Joaquin River water at all percentage additions. Growth response was above the treated tile drainage for all additions except the 20 percent addition, where response equaled that of the 2.94-7.44 mg/l nitrogen category.

Table 7 analyzes the data from the Table 6 column "Treated Agricultural Waste Water." For example, the 1%, 10%, and 20% data in the 0.10-0.17 mg/l category in Table 6, (1.81, 2.70, and 5.94) are located on the first horizontal data line in Table 7. The number 1.00 is again included in the analyses, because of the normalization technique. When compared to Table 6, a finer distinction in the responses is possible in Table 7 because of the smaller variances associated with the treated waste water as compared to the untreated tile drainage.

Response differences at the 1% and 10% additions in Table 7 are similar to those found in Table 6. For the 20% additions of the treated waste water in all nitrogen categories, however, bioassay chlorophyll increases are greater than either the 1% additions or the San Joaquin River controls. This response is unexpected since a 20% addition of treated waste water (containing 0.46-1.00 mg N/l) to San Joaquin River water would increase sample inorganic nitrogen concentration to an average of 0.36 mg N/l. Addition of the 0.10-0.17 mg N/l water would actually dilute the inorganic nitrogen in the San Joaquin River water.

d) Normalized Growth Rates, $\hat{\mu}_b$:

The normalized maximum growth rate data for two experiments are listed in Table 8. The growth rate values are based on two sample readings of the sample fluorescences per day, rather than the usual one-a-day recordings. Thus, they gave a better estimate of the maximum growth rates in this experimental series.

Growth responses for the untreated agricultural tile drain waste water were the only ones exceeding the San Joaquin River control (Table 8). Results of all samples containing untreated agricultural waste water (1%, 5%, 10%, and 20%) fell at the upper end of the multiple range values, although they did not significantly exceed all percentage addition

TABLE 7. - NORMALIZED ALGAL BIOASSAY RESPONSE[g] OF THE TREATED AGRICULTURAL WASTE WATER[h]

NORMALIZED RESPONSE[g]		SAN JOAQUIN RIVER	Percentage Additions to San Joaquin River		
			1%	10%	20%
NO^3-N & NO^2-N in Sample	mg N/l				
Treated Agricultural Waste Water	0.10-0.17	1.00	1.81	2.70	5.94
	0.46-1.00	1.00	1.04	1.88	3.94
	1.45-1.86	1.00	1.29	3.91	9.36
	2.97-7.44	1.00	3.20	13.31	20.98

(g), (h); refer to Table 6.

TABLE 8. - NORMALIZED SUMMARY OF GROWTH RATE RESPONSES, $g_{(\hat{\mu}_b)}$ j

SJR-0 AP-20	BF-1	AP-1	AP-10 BF-10 BF-5	BF-20	AP-5	TD-1	TD-5	TD-20	TD-10
1.00	1.03	1.04	1.08	1.11	1.12	1.28	1.29	1.33	1.36

Code: SJR - San Joaquin River
AP - Algal Pond
BF - Bacterial Filter
TD - Agricultural Tile Drain Waste Water

j. The data are from two experiments in which the test waters were added to San Joaquin River water to make 0, 1, 5, 10, and 20% of total volume. Values connected by underlining do not differ at the 95% confidence level.

values of the algal pond and bacterial filter water. The line does not include the tile drain 1% addition (1.28) indicating that even this small percentage of untreated waste water gives higher algal growth rates than the San Joaquin River control. A substantial overlapping of the multiple range confidence lines occurs between the growth values of the various samples of the treated waste water and the untreated tile drainage. Furthermore, the underlining for the normalized San Joaquin River water (1.00) extends to the algal pond 5% addition response (1.12), signifying no statistical difference in the responses included by the underlining (Table 8).

e) Comparative Algal Pond and Bacterial Filter Nutrient Removal Efficiencies:

A comparison was made of the normalized growth responses of the two nitrogen removal systems. The only growth responses utilized had comparable concentrations of inorganic nitrogen and the same sample dilution for both systems. In Table 9 the normalized response values 6.30 and 6.83 are averages of the ratio of maximum chlorophyll increase in the treated waste water samples to that of the San Joaquin River water. The response values 6.30 and 6.83 are not statistically different ($P < 0.01$).

San Joaquin River Nitrate and Chlorophyll

Table 10 presents initial nitrate concentrations and seasonal chlorophyll concentrations both initially and following incubation.

Initial field chlorophylls for the San Joaquin River samples were low from November through May. They reached a peak of 40.9 μg chl. a/l in July. Little growth occurred during incubation in the laboratory for the samples taken in the summer; no bioassay response was recorded for the July sample when NO_3-N concentrations were the lowest of all those listed (0.04 mg NO_3-N/l). Highest chlorophyll increases were recorded for the 1/28/69 and 2/14/69 samples. These contained the highest initial NO_3-N concentrations, 0.74 and 0.95 mg N/l respectively. Most of the bioassay maximum values for the San Joaquin River water used in controls ranged from 30 to 45 μg chl. a/l. Maximum algal chlorophyll values appear to be equal for many of the sampling months, regardless of whether results were obtained from field or laboratory data.

TABLE 9. - BIOASSAY COMPARISON OF THE NUTRIENT REMOVAL EFFICIENCY OF ALGAL POND AND BACTERIAL FILTER SYSTEMS [k]

	Number of Observations	Normalized Response Mean [g]	F Value
Algal Pond	78	6.30	0.12 [l]
Bacterial Filter	72	6.83	

k. Combined normalized data from inorganic nitrogen content groupings in which both removal systems are represented.

l. The F value would have to be in excess of 3.91 before the normalized response could be considered statistically different ($P > 0.05$).

TABLE 10. - SEASONAL CHLOROPHYLL AND NITRATE VARIATIONS IN THE SAN JOAQUIN RIVER AT ANTIOCH BRIDGE

Date Sample	µg Chl. a/l Initial (Field)	µg Chl. a/l Maximum (Bioassay)	NO_3-N, mg N/l Initial (Field)
1968			
11/14	3.8	34.9	-
12/13	2.0	43.5	0.43
1969			
1/4	1.3	38.8	0.61
1/28	1.5	104.1	0.74
2/14	1.1	69.2	0.95
3/10	1.8	45.6	0.53
4/4	4.2	15.2	0.22
6/18	28.4	38.9	0.18
7/25	40.9	40.9	0.04
8/18	34.8	35.3	0.05
9/9	31.0	33.4	0.08
9/29	32.0	35.7	-
10/20	27.2	35.5	0.09
11/17	20.7	32.1	0.22

CHAPTER V - DISCUSSION

Bioassays were used to evaluate algal growth-promoting properties of treated and untreated agricultural waste water. Samples were diluted by adding them to San Joaquin River water at 1%, 10%, and 20% by volume. The algal growth bioassays were evaluated by measuring chlorophyll concentration maxima and increases. San Joaquin River water collected in the summer contained a high initial concentration of algae and so growth was minimal, thus terminating with only a small increase in chlorophyll concentration. Maximum chlorophyll concentration gives a measure of the total algal mass which can be supported by the sample regardless of whether the conversion of nutrients has occurred in the receiving waters before or after laboratory incubation. Increase in chlorophyll indicates the amount of algal mass formed during the bioassay, however, bioassays that ended with highest increases in chlorophyll also had the highest maximum concentrations of chlorophyll. Bioassay responses can be referred to without specifying either chlorophyll maxima or increases.

The agricultural waste water treated by either the algal pond or anaerobic denitrification systems showed small algal growth responses in the 1% dilution samples. In 10% or 20% dilution samples treated waters promoted algal growth when the removal systems were not performing well. Treated waste water with nitrate concentrations at the lower limit of chemical detectability ($<$0.10 mg N/l) showed growth responses in the 10% and 20% dilution samples similar to those of the San Joaquin River controls.

Instances of substantial algal response occurred when nitrate concentrations were low, indicating other forms of available nitrogen: ammonia and nitrite-nitrogen were present in some samples. There were also examples of little growth in spite of the high nitrate-nitrogen content of the algal pond and bacterial filter samples, suggesting that these systems might have removed other essential plant nutrients or added toxic materials to their effluents.

Samples from the nutrient removal systems almost always gave growth responses below those of untreated agricultural tile drain waste water during the same series of algal assays. This did not necessarily hold true for 1% additions to San Joaquin River water since such small growth differences between samples are more difficult to detect than those at the higher percentage additions.

Normalization of the assay values shows algal response of treated waste water above that of the San Joaquin River control. These data were also grouped to compare experimental responses on the basis of the residual effluent NO_3-N and NO_2-N in the treated tile drain waste water. Statistical evaluation indicates that an effluent nitrogen concentration of less than 2 mg N/l would stimulate algal growth response equal to that of the San Joaquin River control. However, all of the 20% dilutions of treated waste water (Table 7), even those containing less than 2 mg N/l, gave responses above the control. The 20% dilutions of treated waste water in the category containing 0.10-0.17 mg N/l were statistically equivalent to the river water and hence should not increase the bioassay response. There could be several explanations for the anomalous responses in Table 7:

1. The response might be due to limitation by nutrients other than nitrogen.

2. The ammonia in the bacterial denitrification system effluent, which was not differentiated from the organic nitrogen by the analysis, probably contributed significantly to the algal growth.

3. Interpretation of algal growth as based solely upon waste water inorganic nitrogen content might not take all factors into account since the algal pond system obviously would remove algal nutrients other than inorganic nitrogen. Some of these nutrients might be potentially limiting in river/waste water mixtures.

Normalization techniques were also used on growth rate data. These normalized growth rates should not be confused with the absolute growth rates although the numbers obtained are similar. The maximum specific growth rate data ($\hat{u}_b$) is significant in evaluating residence time effects on algal growth. Doubling of the growth rate may more than double the algal mass. This may be critical in areas of short residence time, such as in the transport canals and Carquinez Straits. Observed growth rates during treated waste water bioassays were not statistically different from San Joaquin water. Samples containing untreated agricultural waste water showed growth rates above those of the San Joaquin River controls.

The high and variable nitrate concentration found in the San Joaquin River water which was used for sample dilution might have caused observed growth rate differences in different effluents. Most samples of river water exceeded the 0.06 mg N/l saturation coefficient (K_s) found in

earlier studies (McGauhey, et al., 1968).

Since the detention time of Delta water is sufficient to allow present algal crops to reach their peak (Bain, et al., 1968), nutrient content is presently more important in the San Joaquin Delta than is growth rate. If sediment concentrations become lower and thereby increase light penetration in the Delta-San Francisco Bay System (Krone, 1966), the high bioassay growth rate presently found in San Joaquin River water would indicate the possibility of frequent algal bloom.

Initial bioassays were carried out to determine the effects of inorganic nitrogen and phosphorus compound additions upon the waste water bioassay responses. The addition of nitrate-nitrogen to the algal pond or bacterial filter sample effluents stimulated algal responses so that they equaled the untreated tile drain waste water six out of eight times in samples of comparable dilution. Additions of inorganic phosphorus to treated waste water effluents gave mixed responses. Inadequate algal bioassays were made to provide a statistical evaluation of the results. Two samples of untreated tile drain water responded to the addition of PO_4-P, as did one sample out of five of the bacterial filter systems when PO_4-P was added.

The concomitant use of effluents from two distinct methods of nutrient removal in algal bioassays suggested probable comparison of their efficiency for nutrient removal. Only bioassay data in which both systems had similar concentrations of inorganic nitrogen were compared. It was found that normalized bioassay responses for both systems are almost equal and their differences not statistically significant. Growth rate data comparisons (Table 10) indicated no differences in the algal pond and bacterial filter responses.

The algal bioassay growth of the San Joaquin River water was related to its NO_3-N. Summer and early fall samples exhibited little or no growth between the initial and terminal values made over a period of a week or more. This

lack of algal growth suggests nutrient exhaustion in the Delta waters sampled during the summer and early fall. By contrast, samples from other seasons of the year showed large differences between initial and terminal chlorophyll values. This condition was probably due to available nitrate. Most of the maximum bioassay values recorded were between 30 and 45 μg chl. a/l. This would correspond to approximately 6 - 10mg algae/l dry weight.

APPENDIX A: RESULTS OF THE INDIVIDUAL EXPERIMENTS

Results from the seven experiments utilizing waters collected from June 18 to November 17, 1969, are listed in Tables A1 and A2. The data from each experiment are divided into two sections. One compares all water types for a single percentage dilution (A1.a, A2.a) and the other compares all of the percentage dilutions for a specific water type (A1.b, A2.b).

In comparing the data, underlining was used to indicate values similar to each other at the 95% confidence level. This procedure was allowable because the analyses of variance F values were highly significant for water types, percentages and interaction. In these experiments "interaction" means that increasing the percentage of test waters in San Joaquin River water did not yield comparable increases or decreases in the algal growth for the different test waters.

For example, the values in Tables A1 and A2 should be read in conjunction with the responses for the July 24 experiments in Figures A1 and A2. Both figures show algal chlorophyll concentration changes throughout the conduct of the experiment. It can be seen in Figure A1 that initial chlorophyll values for the different water samples at the beginning of the experiment are not equal and that both the control and the 20% algal pond flasks had no growth. Chlorophyll concentrations decreased from their initial values for these latter two samples.

In Table A1.a, the July 24 experiment shows increases in chlorophyll for the 20% agricultural waste water, bacterial filter, and algal pond samples; 44.0, 18.1 and 0.0 μg chl. a/, respectively. Because each value is statistically different, underlining does not connect them. The maximum chlorophyll concentrations for the same samples as shown in Table A2.a are 80.4, 51.5 and 40.9 μg chl. a/. All values are statistically different at the 95% confidence level.

The algal growth responses for all percentage additions of untreated waste water are graphed in Figure A2. Increases in chlorophyll concentrations, shown in Table A1.b, for the 0, 1, 10 and 20% waste water additions are 0.0, 13.6, 47.0 and 44.0 μg chl. a/, respectively. The highest responses, 47.0 and 44.0 μg chl. a/, are statistically equal. Table A2.a lists the maximum chlorophyll concentrations for the same samples. The maximum values for the 10% and 20% additions, 86.4 and 80.4 μg chl. a/, are statistically equal.

TABLE A1. - BIOASSAY CHLOROPHYLL INCREASES (M)

(a.) PERCENTAGE COMPARISONS

Experiment Date, 1969	µg Chl. a/1 % Additions	AGRICULTURAL WASTE WATER	TREATED AGRICULTURAL WASTE WATER: Bacterial Filter (N)		Algal Pond (N)	
6/18	1%	33.4	19.9		14.6	
	10%	125.7	27.3		32.6	
	100%	0.5	16.5		61.4	
7/24					No.4	
	1%	13.6	1.5		1.5	
	10%	47.0	3.0		1.5	
	20%	44.0	18.1		0.0	
8/18			No.19	No.11	No.15	No.14
	1%	6.2	3.8	0.5	0.9	4.4
	10%	39.6	15.8	0.5	5.2	8.5
	20%	39.3	26.3	3.8	12.2	19.7
9/8				Covered Pond	No.6	No.19
	1%	5.3	5.2	3.8	1.6	0.9
	10%	45.6	16.9	2.4	3.8	31.9
	20%	50.1	45.6	2.4	1.4	36.5
9/29			No.15	No.18	No.5	
	1%	11.1	0.8	0.3	1.9	
	10%	52.3	7.6	5.8	39.7	
	20%	46.1	15.6	15.8	34.6	
10/20			No.10	No.15	No.6	
	1%	27.5	10.7	9.1	17.6	
	10%	64.3	14.1	10.1	35.7	
	20%	63.4	20.4	12.6	58.2	
11/17			No.6		No.14	
	1%	23.1	20.8		18.3	
	10%	36.8	36.5		16.6	
	20%	40.9	19.8		26.7	

(M) Values connected by underlining do not differ at the 95 percent confidence level.
(N) The Firebaugh unit numbers are given when available.

TABLE A1. - BIOASSAY CHLOROPHYLL INCREASES (continued)

(b.) WATER TYPE COMPARISONS

EXPERIMENT DATE, 1969	WATER TYPE	PERCENTAGE ADDITIONS			
		0%	1%	10%	100%
		(μg Chl. a/1)			
6/18	Agricultural Waste Water	10.6	33.4	125.7	0.5
	Algal Pond	10.6	14.6	32.6	61.4
		0%	100%	1%	10%
	Bacterial Filter	10.6	16.5	19.9	27.3
7/24		0%	1%	10%	20%
	Agricultural Waste Water	0.0	13.6	47.0	44.0
	Algal Pond No.4	0.0	1.5	1.5	0.0
	Bacterial Filter	0.0	1.5	3.0	18.1
8/18	Agricultural Waste Water	0.5	6.2	39.6	39.3
	Algal Pond No.14	0.5	4.4	8.5	19.7
	Algal Pond No.15	0.5	0.9	5.2	12.2
	Bacterial Filter No.11	0.5	0.5	0.5	3.8
	Bacterial Filter No.19	0.5	3.8	15.8	26.3
9/8	Agricultural Waste Water	2.4	5.3	45.6	50.1
	Algal Pond No.6	2.4	1.6	3.8	1.4
	Algal Pond No.19	2.4	0.9	31.1	36.5
	Bacterial Filter Covered Pond	2.4	3.8	2.4	2.4
	Bacterial Filter	2.4	5.2	16.9	45.6

TABLE A1. - BIOASSAY CHLOROPHYLL INCREASES - (continued)

(b.) WATER TYPE COMPARISONS - (continued)

EXPERIMENT DATE, 1969	WATER TYPE	PERCENTAGE ADDITIONS			
		1%	0%	10%	20%
			(µg Chl. a/1)		
9/29	Agricultural Waste Water	11.1	3.7	52.3	46.1
	Algal Pond No. 5	1.9	3.7	39.7	34.6
	Bacterial Filter No. 15	0.8	3.7	7.6	15.6
	Bacterial Filter No. 18	0.3	3.7	5.8	15.8
		0%	1%	10%	20%
10/20	Agricultural Waste Water	8.3	27.5	64.3	63.4
	Algal Pond No. 6	8.3	17.6	35.7	58.2
	Bacterial Filter No. 10	8.3	10.7	14.1	20.4
	Bacterial Filter No. 15	8.3	9.1	10.1	12.6
11/17	Agricultural Waste Water	10.5	23.1	36.8	40.9
	Algal Pond No. 14	10.5	18.3	16.6	26.7
		0%	10%	20%	1%
	Bacterial Filter No. 6	10.5	36.5	19.8	20.8

TABLE A2. - BIOASSAY CHLOROPHYLL MAXIMA (P)

(a.) PERCENTAGE COMPARISONS

Experiment Date, 1969	µg Chl. a/1 / % Additions	AGRICULTURAL WASTE WATER	TREATED AGRICULTURAL WASTE WATER: Bacterial Filter (Q)		TREATED AGRICULTURAL WASTE WATER: Algal Pond (Q)	
6/18	1%	60.1	42.5			41.7
	10%	155.6	48.5			63.6
	100%	24.2	28.6			152.3
						No.4
7/24	1%	51.5	42.5			42.5
	10%	86.4	42.5			40.9
	20%	80.4	51.5			40.9
		Agricultural Waste Water	Algal Pond No.14	Bacterial Filter No.19	Algal Pond No.15	Bacterial Filter No.11
8/18	1%	39.4	39.4	37.9	36.4	35.7
	10%	72.8	46.2	48.5	40.2	32.6
	20%	74.3	60.6	59.1	47.8	35.7
			Bacterial Filter			Algal Pond
				Covered Pond	No.6	No.19
9/8	1%	32.6	35.7	31.1	31.1	31.9
	10%	74.1	45.5	31.9	33.4	60.5
	20%	78.9	76.6	34.1	31.9	65.0
			No.15	No.18	No.5	
9/29	1%	43.5	35.0	32.1	32.1	
	10%	83.5	38.2	36.3	76.2	
	20%	75.3	45.6	43.8	66.5	

(P) Values connected by underlining do not differ at the 95 percent confidence level.
(Q) The Firebaugh unit numbers are given when available.

TABLE A2. - BIOASSAY CHLOROPHYLL MAXIMA (continued)

(a.) PERCENTAGE COMPARISONS (continued)

µg Chl. a/l / % Additions / Experiment Date, 1969		AGRICULTURAL WASTE WATER	TREATED AGRICULTURAL WASTE WATER: Bacterial Filter [(N)]		TREATED AGRICULTURAL WASTE WATER: Algal.Pond [(N)]
			No.10	No.15	No.6
10/20	1%	54.6	36.5	35.5	43.5
	10%	90.7	39.0	36.5	60.9
	20%	88.8	45.0	39.7	82.8
			No.6		No.14
11/17	1%	44.5	42.2		39.7
	10%	58.2	58.6		38.1
	20%	62.4	41.8		48.1

TABLE A2. - BIOASSAY CHLOROPHYLL MAXIMA (continued)

(b.) WATER TYPE COMPARISONS

EXPERIMENT DATE, 1969	WATER TYPE	PERCENTAGE ADDITIONS			
		0%	1%	10%	100%
		(µg Chl. a/1)			
6/18	Agricultural Waste Water	38.9	60.1	155.6	24.2
	Bacterial Filter	38.9	42.5	48.5	28.6
	Algal Pond	38.9	41.7	63.6	152.3
		0%	1%	10%	20%
7/24	Agricultural Waste Water	40.9	51.5	86.4	80.4
	Bacterial Filter	40.9	42.5	40.9	40.9
	Algal Pond No.4	40.9	42.5	42.5	51.5
8/18	Agricultural Waste Water	34.9	39.4	72.8	74.3
	Bacterial Filter No.11	34.9	35.7	32.6	35.7
	Bacterial Filter No.19	34.9	37.9	48.5	59.1
	Algal Pond No.15	36.4	36.4	40.2	47.8
	Algal Pond No.14	36.4	39.4	46.2	60.6
9/8	Agricultural Waste Water	33.9	32.6	74.1	78.9
	Bacterial Filter Covered Pond	34.1	31.1	31.9	34.1
	Bacterial Filter	34.9	35.7	45.5	76.6
	Algal Pond No.6	34.1	31.1	33.4	31.9
	Algal Pond No.19	33.9	31.9	60.5	65.0

TABLE A2. - BIOASSAY CHLOROPHYLL MAXIMA (continued)

(b.) WATER TYPE COMPARISONS - (continued)

EXPERIMENT DATE, 1969	WATER TYPE	PERCENTAGE ADDITIONS			
		1%	0%	10%	20%
		(µg Chl. a/1)			
9/29	Agricultural Waste Water	43.5	35.7	83.5	75.3
	Bacterial Filter No.15	35.0	35.7	38.2	45.6
	Bacterial Filter No.18	32.1	35.7	36.3	43.8
	Algal Pond No.5	32.1	35.7	71.2	66.5
		0%	1%	10%	20%
10/20	Agricultural Waste Water	35.5	54.6	90.7	88.8
	Bacterial Filter No.10	35.7	36.5	39.9	45.0
	Bacterial Filter No.15	35.5	35.5	36.5	39.7
	Algal Pond No.6	35.0	43.5	60.9	82.8
11/17	Agricultural Waste Water	30.6	44.5	58.2	62.4
	Algal Pond No.14	30.6	39.7	38.1	48.1
		0%	1%	20%	10%
	Bacterial Filter No.6	30.6	42.2	41.8	58.6

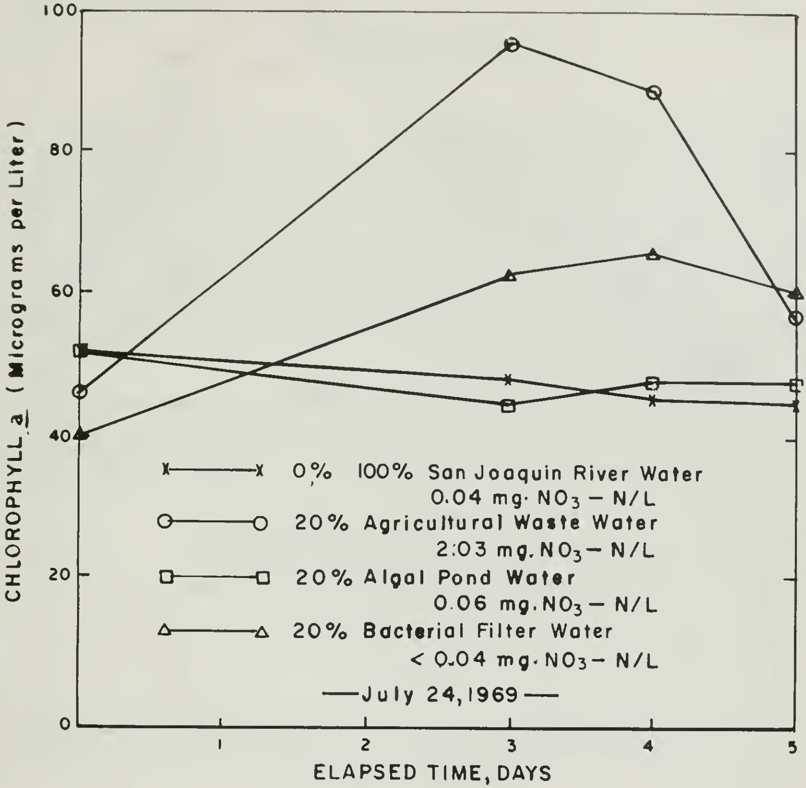

Figure A1. Algal Growth Response of San Joaquin River Water and Mixed Samples

AGRICULTURAL WASTE WATER STUDIES
SAN JOAQUIN VALLEY, CALIFORNIA

ENVIRONMENTAL PROTECTION AGENCY
REGION IX
SAN FRANCISCO, CALIFORNIA

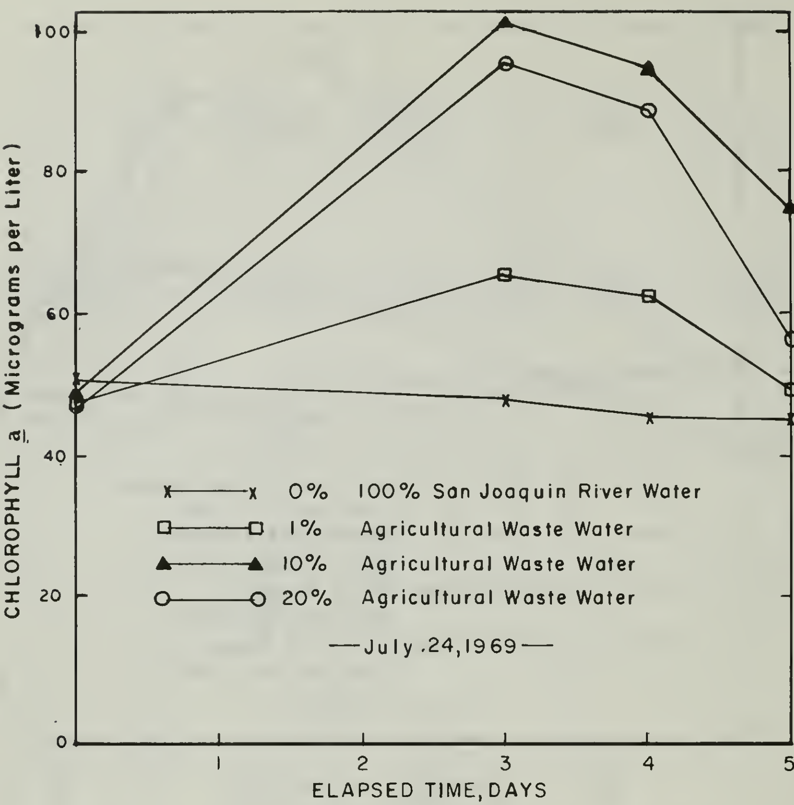

Figure A2 Algal Growth Response of San Joaquin River Water with Added Agricultural Waste Water.

AGRICULTURAL WASTE WATER STUDIES
SAN JOAQUIN VALLEY, CALIFORNIA

ENVIRONMENTAL PROTECTION AGENCY
REGION IX
SAN FRANCISCO, CALIFORNIA

APPENDIX B: ALGAL SPECIES AND NUMBERS

Algae were identified and counted at the beginning and end of several experiments. Personnel from the California Department of Water Resources identified and counted algae in Firebaugh water collected on September 9, 1969. Proportional counts of the algal species were made by algal groups (Table B1). The proportional counts listed in Table B1 show that diatoms comprised more than 74% of the species at the beginning and end of the experiment in San Joaquin River waters, in the bacterial filter, and in the pond water additions, with or without supplemental inorganic nitrogen.

The addition of phosphorus to any sample shifted the population percentages from diatoms to green algae. Inorganic nitrogen and phosphorus were also added to some water samples.

TABLE B1.- THE PROPORTIONAL COUNTS OF ALGAE FOUND INITIALLY AND AT THE TERMINATION OF THE BIOASSAY EXPERIMENTS

Algal Group / Percent of Total No.	San Joaquin River				Algal Pond				Bacterial Filter			Agricultural Waste Water	
	Initial	Terminal	6.7 mg N/1	6.7 mg N/1 6.7 mg P/1 [r]	2% AP	5% AP 6.7 mg N/1	5% AP 6.7 mg N/1 3.3 mg P/1	5% AP 6.7 mg N/1 13.2 mg P/1	2% BF	5% BF 6.7 mg N/1 3.3 mg P/1	5% BF 6.7 mg N/1 6.7 mg P/1	10%	5% 13.2 mg P/1
Greens	6	6	10	56	2	15	69	68	1	51	53	12	55
Blue-greens	-	13	1	-	3	1	21	3	13	39	12	-	1
Diatoms	86	75	86	23	89	74	6	21	84	7	28	83	35
Flagellates	8	6	3	21	6	10	4	8	2	3	7	5	9

r. These are additions in waste water concentrations.

APPENDIX C: CELL COUNT AND CHLOROPHYLL CONCENTRATION

Algal cells were counted and chlorophyll concentrations were measured at the beginning and end of four experiments conducted on June 18, 1969; July 24, 1969; August 18, 1969; and November 17, 1969. Replicate flasks were combined and a single count made of the algal cell concentrations numbers in the combined samples. The correlation coefficient between the algal numbers and the sample chlorophyll concentration is 0.69, and is highly significant statistically ($p<0.01$). The cell count versus chlorophyll data are shown in Figure C1.

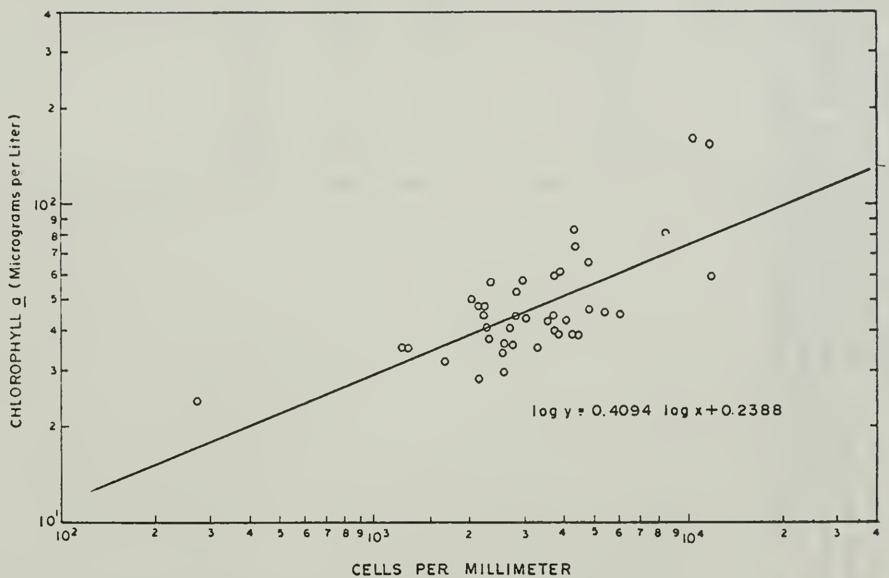

Figure C1. Cell Count vs Chlorophyll a Concentrations, Initial and Terminal Measurements.

APPENDIX D
BIOASSAY NITROGEN RESPONSES

Nitrate determinations were made of each concentration at the beginning and end of all experiments except the first two (June 18 and July 24). It was expected that algal growth would decrease the NO_3-N concentrations at the end of the experiment period. This supposition proved true; most samples had a terminal nitrate concentration of 0.10 mg N/l or less (Table D1).

Higher terminal NO_3-N concentrations were found when the initial concentration of nitrate was high. For example, in the 20% agricultural waste water flasks of August 18, the NO_3-N was 1.88 mg N/l at the beginning and 1.00 mg N/l at the end. Nitrate-nitrogen decreased 0.88 mg/l for this particular sample. In the 10% agricultural waste water sample of September 29th the maximum decrease in NO_3-N was 1.32 mg/l.

Slight increases in nitrate also occurred during some experiments. For example, NO_3-N for all concentrations increased in the anaerobic covered pond of September 8th although there was no detectable NO_3-N in the original covered pond sample (Table 3). There was however 1.13 mg/l of organic nitrogen found in the sample. Since no discernable growth of algae occurred using this water, NO_3-N increases may have resulted from bacterial oxidation of the organic nitrogen. In some of the experiments an increase in nitrate may have resulted from oxidation of nitrite to nitrate. Many of the samples listed in Table 2 had a high concentration of nitrite-nitrogen.

The increase in chlorophyll and the decrease in NO_3-N are plotted in Figure D1. The correlation coefficient ($r = 0.82$) between the two variables is highly significant, the regression line having the equation:

$$\mu g\ N/l = 11.6x\ (\mu g\ chl.\ \underline{a}/l) - 15.0$$

This formula gives a ratio of nitrate-nitrogen decrease to chlorophyll increase of approximately 11:1. The ratio closely agrees with the organic nitrogen-chlorophyll ratio of Yentsch and Vaccaro (1958) for nitrogen enriched cultures, (7:1 to 10:1) and the ratio found by Manny (1969) which was 6:1 to 12:1. These ratios referred to fixed organic nitrogen while ratios used in this project were based on the assumption that the nitrate-nitrogen was metabolized into an organic form.

TABLE D1. NITRATE CONCENTRATIONS IN TEST WATERS BEFORE AND AFTER BIOASSAY

		mg/1 NO_3-N		
Experiment Dates- 8/18/69 to 8/25/69		Beginning	End	Decrease
San Joaquin River	100%	0.05	0.10	0
Agricultural	1%	0.14	0.08	0.06
Waste Water	10%	0.96	0.30	0.66
	20%	1.88	1.00	0.88
Algal	1%	0.06	0.08	0
Pond No. 14	10%	0.18	0.08	0.10
High-rate	20%	0.32	0.08	0.24
Algal	1%	0.08	0.10	0
Pond No. 15	10%	0.36	0.08	0.28
Low-rate	20%	0.66	0.08	0.58
Bacterial	1%	0.05	0.10	0
Filter No. 11	10%	0.09	0.08	0.10
High-rate	20%	0.14	0.08	0.04
Bacterial	1%	0.10	0.10	0
Filter No. 19	10%	0.56	0.09	0.47
Low Rate	20%	1.06	0.11	0.95
Experiment Dates - 9/8/69 to 9/16/69				
San Joaquin River	100%	0.08	0.05	0.03
Agricultural	1%	0.16	0.07	0.09
Waste Water	10%	0.80	0.52	0.28
	20%	2.30	1.60	0.70
Algal	1%	0.08	0.11	0
Pond No. 6	10%	0.07	0.12	0
	20%	0.08	0.07	0.01
Algal	1%	0.08	0.10	0
Pond No. 19	10%	0.30	0.09	0.21
	20%	0.64	0.16	0.48
Anaerobic	1%	0.06	0.08	0
Covered	10%	0.06	0.07	0
Pond	20%	0.05	0.08	0
Bacterial	1%	0.06	0.07	0
Filter	10%	0.06	0.05	0.01
	20%	0.05	0.10	0

BLE D1. NITRATE CONCENTRATIONS IN TEST WATERS BEFORE AND AFTER BIOASSAY - (continued)

		mg/l NO_3-N		
Experiment Dates - 9/29/69 to 10/6/69		Beginning	End	Decrease
San Joaquin River	100%	0.03	0.03	0
Agricultural	1%	0.18	0.03	0.15
Waste Water	10%	2.1	0.78	1.32
	20%	3.8	2.7	1.1
Algal	1%	0.05	0.03	0.02
Pond No. 5	10%	0.47	0.03	0.45
	20%	0.84	0.14	0.70
Bacterial	1%	0.03	0.04	0.
Filter	10%	0.08	0.04	0.04
No. 15	20%	0.20	0.03	0.17
Bacterial	1%	0.03	0.03	0
Filter	10%	0.08	0.03	0.05
No. 18	20%	0.11	0.03	0.08
Experiment Dates - 10/20/69 to 10/27/69				
San Joaquin River	100%	0.12	0.19	0
Agricultural	1%	0.30	0.47	0
Waste Water	10%	1.6	0.75	0.85
	20%	3.6	3.0	0.6
Algal	1%	0.14	0.03	0.11
Pond No. 6	10%	0.30	0.03	0.27
	20%	0.51	0.03	0.48
Bacterial	1%	0.10	0.03	0.07
Filter	10%	0.12	0.03	0.09
No. 10	20%	0.12	0.03	0.09
Bacterial	1%	0.11	0.03	0.08
Filter	10%	0.14	0.03	0.11
No. 15	20%	0.18	0.03	0.15
Experiment Dates - 11/17/69 to 11/24/69				
San Joaquin River	100%	0.22	0.03	0.19
Agricultural	1%	0.45	0.04	0.41
Waste Water	10%	3.2	2.4	0.80
	20%	4.1	3.7	0.4
Algal	1%	0.22	0.04	0.18
Pond No. 14	10%	0.29	0.03	0.26
	20%	0.37	0.03	0.34
Bacterial	1%	0.38	0.04	0.34
Filter No. 6	10%	1.5	1.0	0.50
	20%	3.2	-	-

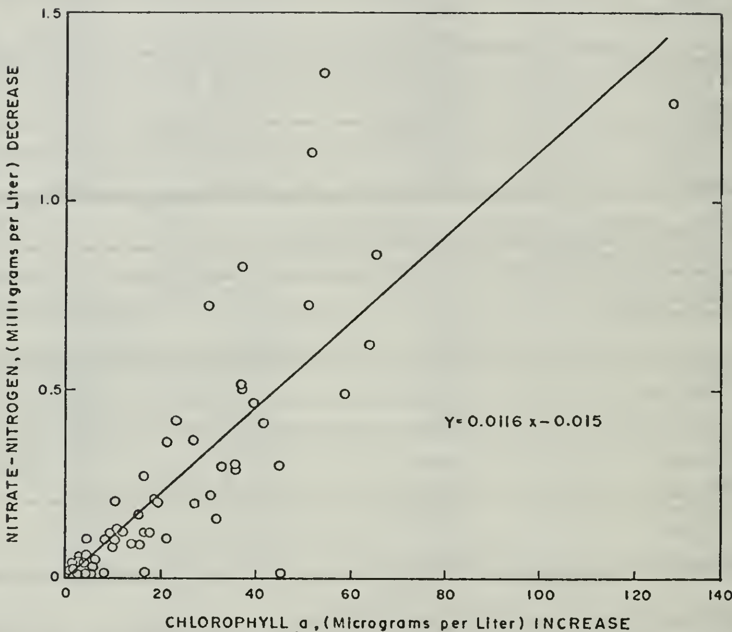

Figure D1. Bioassay Decrease in Nitrate-Nitrogen as a Function of Chlorophyll Increases..

Figure D2 shows the log of the chlorophyll concentration maximums as a function of the log of the original NO_3-N contained in the cultures. The correlation is highly significant ($r = 0.77$), the equation of the line being:

$$\log(\mu g \text{ chl. } \underline{a}/) = 0.2003 (\log \mu g \text{ N/l}) + 1.195$$

The ratio of total initial inorganic nitrogen to maximum chlorophyll in the culture is approximately 16:1.

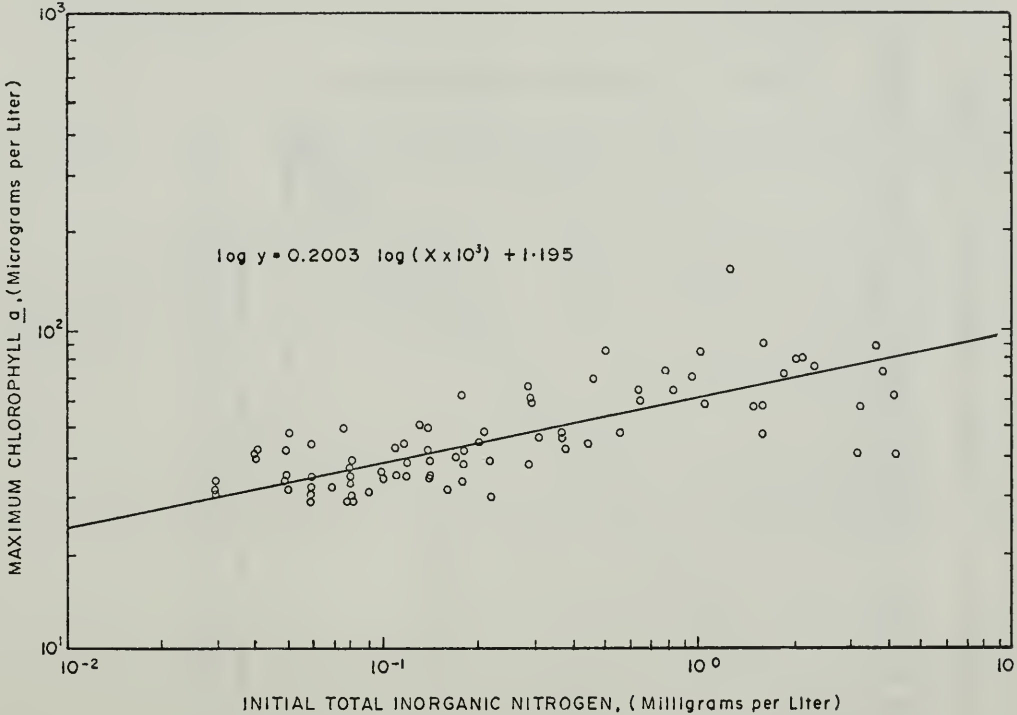

Figure D2. Bioassay Chlorophyll Maximum as a Function of Total Inorganic Nitrogen (NO_3–N plus NO_2–N).

ACKNOWLEDGEMENTS

The following list includes the personnel actively involved in the algal bioassay project.

Firebaugh Project under the Direction of

Percy St. Amant..................Engineer, Environmental Protection Agency
Louis A. Beck....................Engineer, California Dept. of Water Resources
Donald Swain.....................Engineer, U.S. Bureau of Reclamation

Consultants to the Project

Dr. Perry L. McCarty.............Stanford University, Palo Alto
Dr. William J. Oswald............University of California, Berkeley
Dr. Clarence G. Golueke..........University of California, Berkeley

Algal Bioassays of Agricultural Waste Water Conducted by

Dr. Milton G. Tunzi..............Limnologist, Environmental Protection Agency

Assisted by

Albert Katko.....................Aquatic Biologist, EPA
Richard C. Bain, Jr..............Sanitary Engineer, EPA
James E. Thoits..................Biological Technician, EPA
Ella M. McGehee..................Physical Science Aide, EPA
Dorothy M. White.................Physical Science Aide, EPA
Margaret Y. Chu..................Chemist, EPA
Mustafa M. Salma.................Chemist, EPA
David R. Wood....................Supervisory Chemist, EPA
Kathleen Shimmin.................Supervisory Microbiologist, EPA
Gary Varney......................Biologist, California Dept. of Water Resources

Report Prepared by

Dr. Milton G. Tunzi..............Limnologist, Environmental Protection Agency

LIST OF REFERENCES

Bain, R.C., Jr. 1969. Algal Growth Assessments by Fluorescence Techniques: Proceedings of the Eutrophication--Biostimulation Assessment Workshop, Federal Water Quality Administration, Corvallis, Oregon.

Bain, R.C., Jr., H.E. Pintler, A. Katko, and R.F. Minnehan. 1968. Nutrients and Biological Response: San Joaquin Master Drain, Appendix, Part C, Federal Water Quality Administration, Southwest Region, San Francisco, 117p.

FWPCA Methods for Chemical Analysis of Water and Wastes. November 1969. Federal Water Quality Administration, Analytical Quality Control Laboratory, Cincinnati, Ohio, 280p.

Krone, R.B. 1966. Predicted Suspended Inflows to the San Francisco Bay System, Prepared for the Federal Water Pollution Control Administration, Southwest Region, San Francisco.

Manny, B.A. 1969. The Relationship between Organic Nitrogen and the Carotenoid to Chlorophyll A Ratio in Five Freshwater Phytoplankton Species: Limnology and Oceanography, V. 14, p. 69-79.

McGaughey, P.H., G.A. Rohlick, E.A Pearson, M. Tunzi, A. Adinarayana, and E.J. Middlebrooks. 1968. Eutrophication of Surface Waters-Lake Tahoe: Bioassay of Nutrient Sources. LTAC, FWPCA Progress Report for Grant No. WPD 48-01 (R 1), May.

Provisional Algal Assay Procedures. 1969. Joint Industry-Government Task Force on Eutrophication, P.O. Box 3011, Grand Central Station, New York, 43p.

Sword, B. R. 1970. Denitrification of Agricultural Tile Drainage in Anaerobic Filters and Ponds, in this series.

Yentsch, C.S. and R.F. Vaccaro. 1958. Phytoplankton Nitrogen in the Oceans: Limnology and Oceanography, V. 3, p. 443-448.

PUBLICATIONS

SAN JOAQUIN PROJECT, FIREBAUGH, CALIFORNIA

1968

"Is Treatment of Agricultural Waste Water Possible?"
Louis A. Beck and Percy P. St. Amant, Jr. Presented at Fourth International Water Quality Symposium, San Francisco, California, August 14, 1968; published in the proceedings of the meeting.

1969

"Biological Denitrification of Wastewaters by Addition of Organic Materials"
Perry L. McCarty, Beck, and St. Amant, Jr. Presented at the 24th Annual Purdue Industrial Waste Conference, Purdue University, Lafayette, Indiana. May 6, 1969.

"Comparison of Nitrate Removal Methods"
Beck, St. Amant, and Thomas A. Tamblyn. Presented at Water Pollution Control Federation Meeting, Dallas, Texas. October 9, 1969.

"Effect of Surface/Volume Relationship, CO_2 Addition, Aeration, and Mixing on Nitrate Utilization by Scenedesmus Cultures in Subsurface Agricultural Waste Waters"
Randall L. Brown and James F. Arthur. Proceedings of the Eutrophication-Biostimulation Assessment Workshop, Berkeley, California. June 19-21, 1969.

"Nitrate Removal Studies at the Interagency Agricultural Waste Water Treatment Center, Firebaugh, California"
St. Amant and Beck. Presented at 1969 Conference, California Water Pollution Control Association, Anaheim, California, and published in the proceedings of the meeting. May 9, 1969.

"The Anaerobic Filter for the Denitrification of Agricultural Subsurface Drainage"
Tamblyn and B. R. Sword. Presented at the 24th Annual Purdue Industrial Waste Conference, Lafayette, Indiana. May 5-8, 1969.

1969 (Continued)

"Research on Methods of Removing Excess Plant Nutrients from Water"
St. Amant, Beck. Presented at 158th National Meeting and Chemical Exposition, American Chemical Society, New York, New York. September 8, 1969.

"Nutrients in Agricultural Tile Drainage"
W. H. Pierce, Beck, and L. R. Glandon. Presented at the 1969 Winter Meeting of the American Society of Agricultural Engineers, Chicago, Illinois. December 9-12, 1969.

"Treatment of High Nitrate Waters"
St. Amant, McCarty. Presented at Annual Conference, American Water Works Association, San Diego, California,. May 21, 1969. American Water Works Association Journal, Vol. 61, No. 12. December 1969, pp. 659-662.

The following papers were presented at the National Fall Meeting of the American Geophysical Union, Hydrology Section, San Francisco, California. December 15-18, 1969. They are published in Collected Papers Regarding Nitrates in Agricultural Waste Water. USDI, FWQA, #13030 ELY December 1969.

"The Effects of Nitrogen Removal on the Algal Growth Potential of San Joaquin Valley Agricultural Tile Drainage Effluents"
Brown, Richard C. Bain, Jr., and Milton G. Tunzi.

"Harvesting of Algae Grown in Agricultural Wastewaters"
Bruce A. Butterfield and James R. Jones.

"Monitoring Nutrients and Pesticides in Subsurface Agricultural Drainage"
Glandon and Beck.

"Combined Nutrient Removal and Transport System for Tile Drainage from the San Joaquin Valley"
Joel C. Goldman, Arthur, William J. Oswald, and Beck.

"Desalination of Irrigation Return Waters"
Bryan R. Sword.

"Bacterial Denitrification of Agricultural Tile Drainage"
Tamblyn, McCarty, and St. Amant.

"Algal Nutrient Responses in Agricultural Wastewater"
Arthur, Brown, Butterfield, and Goldman.

1 Accession Number	2 Subject Field & Group	
	05D	SELECTED WATER RESOURCES ABSTRACTS INPUT TRANSACTION FORM

5 Organization

Environmental Protection Agency, Region IX
Water Quality Office
100 California Street, San Francisco CA 94111

6 Title

The Effects of Agricultural Waste Water Treatment on Algal Bioassay Response

10 Author(s)	16 Project Designation
TUNZI, Milton G.	13030 ELY
	21 Note: Available from: Environmental Protection Agency Region IX, 100 California Street San Francisco CA 94111

22 Citation

Agricultural Waste Water Studies
Report No. 13030 ELY 8/17-9
Pages: 59, Figures: 8, Tables: 14, References: 9

23 Descriptors (Starred First)

* Eutrophication
* Bioassay
* Denitrification , Nitrates, Nitrogen
* Fluorometry
* Tile Drains

25 Identifiers (Starred First)

* Algal Blooms - control, bioassay - algal, chlorophyll, denitrification, fluorometry, tile drainage

27 Abstract

Laboratory bioassay experiments were performed to test the effect on algal growth of agricultural waste water before and after the waste water had been subjected to two different nitrogen removal processes. The waste waters were added in various percentages to San Joaquin River Delta water for bioassay. The algal growth throughout time was monitored by chlorophyll fluorescence techniques. The fluorescence measurements showed logarithmic growth similar to the type usually observed in the Delta Water over the vernal growth period.

The laboratory experiments gave positive statistical evidence that the untreated agricultural waste water would promote substantial algal growth above that of the San Joaquin River controls. Both nitrogen removal processes were equally effective in lowering the algal growth to that of the Delta water controls as long as the nitrate-nitrogen level in each removal system had been lowered to approximately 2 mg N/l, or less.

Abstractor	Institution

ENVIRONMENTAL PROTECTION AGENCY
Publications Distribution Section
Route 8, Box 116, Hwy. 70, West
Raleigh, North Carolina 27607

Official Business

CLEAN WATER

POSTAGE AND FEES PAID

ENVIRONMENTAL PROTECTION AGENCY

If your address is incorrect, please change on the above label; tear off; and return to the above address.
If you do not desire to continue receiving this technical report series, CHECK HERE ☐ ; tear off label, and return it to the above address.